



Resilient populations of root fungi occur within five tomato production systems in southeast Florida

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ABSTRACT

Farming practices are known to impact arbuscular mycorrhizal (AM) fungi and other soil microbial communities in agroecosystems. The effects of divergent land management strategies on the incidence and infectivity of AM and other fungal root endophytes were evaluated in a 5-year tomato (*Lycopersicon esculentum* Mill.) cropping systems study. Two of the five treatments utilized farming practices considered detrimental to AM fungal populations, including the tillage-mediated elimination of vegetation and soil fumigation. The remaining three treatments used practices thought to be more conducive to the presence of AM fungi, including organic production methods, bahiagrass pasture and undisturbed weed fallow. In years four and five of the study tomato roots and rhizosphere soil were collected. Roots were examined for colonization by AM and other fungal root endophytes, and rhizosphere soil was assayed to measure the amount of infective inoculum present based on maize (*Zea mays* L.) seedling infection. Tomato roots and rhizosphere soil were also analyzed for the AM fungal fatty acid biomarker 16:1 ω 5c. Sudangrass (*Sorghum sudanense* (Piper) Stapf) trap cultures were initiated using field soil to assess the diversity of AM fungal spore morphotypes. Soil disturbance and phosphorus (P) levels had the greatest influences on AM fungal infectivity and abundance. All plots had high levels of available soil P, resulting in low levels of colonization across treatments. Bahiagrass (*Paspalum notatum* Flugge) pasture was the only treatment without repeated, intensive soil mixing, and had the highest level of field root colonization by AM fungi. Field roots were more heavily colonized by other fungal endophytes than by AM fungi in all treatments. Tomato roots from organic plots were apparently unique in encouraging colonization by fungi that appeared to be *Microdochium bolleyi* (R. Sprague) de Hoog & Herm.-Nijh. Infection by AM and other fungal root endophytes were positively correlated in all studies. Flooding and a shortened growing season likely contributed to reduced infection potentials in all treatments except for organic plots in year five compared to year four. Areas of high disturbance from frequent tillage had the lowest levels of primary inoculum, but recovery to levels comparable to less disturbed treatments occurred after a single season of host root growth. Diversity of AM fungal morphotypes was typical of agricultural fields, with at least 10 spore morphotypes present across treatments; *Glomus* spp. were the dominant spore type recovered in all treatments.

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1. Introduction

Arbuscular mycorrhizal (AM) fungi form symbioses with most herbaceous plants, are present in a wide range of terrestrial environments, and perform a variety of functions in their associations within natural and agroecosystems (Douds and

Millner, 1999). These mycorrhizal associations are believed to be capable of making a significant positive contribution to plant health as well as positively affecting soil quality (Douds and Millner, 1999; Jeffries et al., 2003; Johansson et al., 2004). However, experimental evidence suggests that field conditions common in industrialized agriculture may constrain colonization by natural AM fungal communities (Kabir et al., 1999; Oehl et al., 2004; Gosling et al., 2006). Widespread management practices used to meet production goals such as phosphorus (P) fertilization (Thingstrup et al., 1998); tillage-mediated soil disturbance (Borie et al., 2006); application of pesticides (Trappe et al., 1984); and soil fumigation (Klose et al., 2006) are potentially detrimental to populations of AM fungi. Because of the widespread use of and

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production benefits gained from practices that may be deleterious to AM fungal populations, as well as the carbon cost inherent to plants in interactions with these obligate biotrophs fungi, it has been questioned as to whether or not AM fungi should be considered in the management decisions of high-input agricultural systems (Ryan and Graham, 2002).

In addition to the AM fungi, there are many other fungal endophytes widely capable of colonizing the root cortex of plants; some of which infect the stele region (Yu et al., 2001; Barrow, 2003). Definitions for the term endophyte vary and different uses of the word can be controversial (Wennström, 1994; Sieber, 2002). In this study endophytes are defined as those fungi that exist within plant roots without eliciting visible symptoms of disease. Recognizing that AM fungi fit this definition, when the term endophyte is used in this work it will be qualified when referring to fungi not within the phylum Glomeromycota. These endophytes may function as obligate or facultative biotrophs across plant-microbe interactions ranging from mutualism to parasitism or pathogenicity (Morton, 1998; Sylvia and Chellemi, 2001; Sieber, 2002). Some *formae speciales* of *Fusarium oxysporum* Schltdl.:Fr., for example, are pathogenic on select plant taxa but are seemingly innocuous or even beneficial in associations with other plants. It is probable that all plants form associations with one or more taxa of fungal root endophyte (Sieber, 2002).

The possible functional role of non-AM fungal root endophytes in agroecosystems has received substantial interest, but information regarding their status under contrasting agricultural management is sparse. The value of this group of organisms as biological indicators of soil quality and health may be significant, and their impact on ecosystem functioning deserves more study (Hill et al., 2000; van Bruggen and Semenov, 2000; Chellemi and Porter, 2001). Considerable information exists detailing the impacts of agricultural practices on AM fungal communities (Kabir et al., 1999; Galvez et al., 2001; Oehl et al., 2004; Gryndler et al., 2006), but less is known of the ability and extent to which these communities recover if land management practices are changed, or of long-term adverse changes in fungal infectivity due to farming strategies such as annual soil fumigation.

The main goal of this study was to characterize the impacts that varied management practices have upon fungal root endophytes in agroecosystems. This objective included elucidating how industrialized agricultural practices affected the survival, infectivity and occurrence of AM and other fungal root endophytes. In addition, it was important to determine the degree to which farming strategies that have been considered more amenable to the persistence and functioning of AM fungi were able to influence their activity after

long-term exposure to intensive tomato (*Lycopersicon esculentum* Mill.) production.

2. Materials and methods

2.1. Field experiment site and design

Data were collected from the USDA, ARS cropping systems project at Header Canal near Fort Pierce, Florida (27.22° north and 80.29° west) in the southeastern U.S.A. Mean annual precipitation is 1308 mm with 56% of this amount occurring between June and September. The maximum and minimum temperatures reached during the 5-year experiment were 35.7 and −0.3 °C, respectively. Previously, the experimental field site was a commercial tomato farm subjected to 10 consecutive years of conventional production practices that included soil fumigation with methyl bromide and chloropicrin. The soil type is a Pineda fine sand (loamy, siliceous, hyperthermic, Arenic Glossaqualfs), with a soil texture of 94–3–3% sand–silt–clay, and soil bulk density of 1.4 g cm^{−3} at a 0–30 cm sample depth.

Treatments representing five divergent land management practices typical of agricultural production in the region were established in a randomized complete block design, with six replications. Each replicate plot was 0.16 ha (plots were 68.5 m × 23 m). Treatments were (1) 'conventional tomato production'; (2) 'disk fallow'; (3) 'weed fallow'; (4) 'organic'; and (5) 'bahiagrass' (Table 1). Tomatoes in all treatments except the bahiagrass (*Paspalum notatum* Flugge) system were grown in raised beds with plastic mulch.

Conventional tomato production included soil fumigation with a 62:35 formulation of 1,3-dichloropropene and chloropicrin and the herbicides trifluralin (a,a,a-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine) and napropamide (((R,S)-N,N-diethyl-2-(1-naphthyl-oxy)propionamide). In the first 3 years of the study, fertilizer was spread and incorporated into beds. A similar procedure was followed in the fourth and fifth years, except that most fertilizer was applied via drip irrigation at regular intervals during the crop production period. Irrigation tubing was placed 5 cm beneath the soil and 25 cm from the edge of beds.

In organically managed plots, amendments were applied annually between 19 June and 10 July and immediately incorporated into soil using a 30 cm leveling disk. Broiler litter (BL) was obtained from commercial poultry production houses in Live Oak, Florida and consisted of a mixture of pine shavings and manure. The BL was dry stock, and was aged between 6 and 12 weeks in static row piles with an ash content below 25%. Urban

Table 1

Summary of land management strategies of the 5-year cropping systems study.^a

Treatment	Year	January–June	July	August–December
Bahia pasture	1	Plant seed, synthetic fertilizer	Periodic mowing	Periodic mowing
	2–3	Periodic mowing	Periodic mowing	Periodic mowing
	4–5	Periodic mowing	Periodic mowing	Strip tillage, synthetic fertilizer, transplant tomato
Conventional	1–5	Undisturbed fallow	Cultivation	Fumigation, synthetic fertilizer, raised plastic-mulched beds, transplant tomato
Disk fallow	1–3	Continuous tillage	Continuous tillage	Continuous tillage
	4–5	Continuous tillage	Cultivation	Synthetic fertilizer, raised plastic-mulched beds, transplant tomato
Organic	1–3	Cover crop (Japanese millet)	Soil amendments (BL and UPD), cultivation	Cover crop (Sunn Hemp)
	4–5	Cover crop (Japanese millet)	Soil amendments (BL and UPD), cultivation	Soil solarization, raised plastic-mulched beds, transplant tomato
Weed fallow	1–3	Undisturbed fallow	Undisturbed fallow	Undisturbed fallow
	4–5	Undisturbed fallow	Cultivation	Synthetic fertilizer, raised plastic-mulched beds, transplant tomato

^a This table shows the southern half of plots, which had a 3-year transition prior to tomato production. Northern halves of plots had a 4-year transitional phase.

plant debris (UPD) obtained from commercial landfills was passed through 2.5 cm mesh screens, tub ground and partially composted prior to application. Fresh weight application rates ranged from 20.8 to 25.3 Mg ha⁻¹ for BL and 60.3–75.3 Mg ha⁻¹ for UPD. Sunn hemp (*Crotalaria juncea* L.) was planted each year at a seeding rate of 33.6 kg ha⁻¹ several weeks after amendments were applied. In February, the sunn hemp was mowed and incorporated into the soil. Japanese millet (*Echinochloa crus-galli frumentacea* (Link.) W. Wight) was planted in March at a seeding rate of 21 kg ha⁻¹.

To establish bahiagrass (cv. Argentine) pasture, 56–0–31 kg ha⁻¹ of N–P₂O₅–K₂O and 22.4 kg ha⁻¹ of seed was soil incorporated in August of the first year. In April of the second year, N–P₂O₅–K₂O was added again at the same rate, and plots were over-seeded at a rate of 9 kg ha⁻¹. The bahiagrass was mowed regularly during the growing season. Instead of raised beds, tomatoes were planted into a 46-cm strip tilled into the bahiagrass, while grass on either side of the rows was managed through mowing and use of the herbicide sethoxydim (2 [1(ethoxyimino) butyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen-1-one) (Chellemi et al., 1999).

The disk fallow treatment consisted of tillage conducted at frequent intervals to maintain the plots free of vegetation. Soil was tilled to a 15 cm depth using 30 cm offset disks, and followed by a cultivator with swept back shanks attached to rolling baskets. In the weed fallow treatment, plots were left undisturbed and weed communities were allowed to establish. Tomato production began in year four following disking, after which the plots in both of these treatments utilized the practices used in the conventional system, although no soil fumigants were used.

The conventional treatment was the only system without a 3-year transitional stage, with tomato production continuing during every growing season. In year four of all other treatments in this project, tomato production was initiated in the southern half of each plot, and in year five a tomato crop was grown on both halves. During the initiation of tomato crop production in year five of the study, two hurricanes struck the area of Florida where the farm site was located. Rainfall totals exceeded 50 cm during the month of September, resulting in widespread flooding. Five-week-old tomato seedlings (cv. Florida 91) were transplanted into plastic mulched beds in September of the first 4 years of the experiment, but in year five planting was delayed approximately a month due to the hurricanes and the resultant field conditions. The appearance, vigor and growth of tomato plants following transplanting appeared normal in all 5 land management strategies.

2.2. Sample collection and processing

Soil samples collected in years four and five of the project were used to assess the fungal infection potential (IP), and to establish pot cultures to induce sporulation and identify native AM fungi. Sampling took place 105 days after transplanting, 4 days after final harvest in year four, and 71 days after transplanting, 1 day after the first harvest in year five. Samples were taken directly beneath tomato and other targeted plants, with thirty cores taken from each plot to a depth of 20 cm. To approximate the rhizosphere of the plant, for each sample that was taken, the probe entered at the base of a plant and was angled slightly to capture a substantial portion of the root system along with the soil. In addition to samples from beneath tomato plants, cores were also taken from the sunn hemp cover crop in the organic system, from the bahia sod in the bahiagrass system and from ragweed (*Ambrosia artemisiifolia* L.), an abundant weed species across weed fallow plots. Soil from each plot was bulked and a portion was sent to Waters Agricultural Laboratories in Camille, GA for fertility analysis in both years four and five. The Mehlich-1 extraction procedure was used to determine levels of available nutrients.

Root samples were collected from tomato and other targeted plants to assess colonization by AM and other fungal root endophytes. Four tomato plants from each plot were dug from the center 100 plants of middle rows. Roots from 120 tomato plants were analyzed in year four (southern half of plots, four plants × 30 plots), and from 240 plants in year five (both halves of plots, 8 plants × 30 plots). In year four, sunn hemp, ragweed and bahiagrass plant roots were also sampled in their respective treatment systems (see above). Roots in both year four and five were placed in paper bags and dried at 70 °C, after which fine roots were sampled for staining.

2.3. Observation of root-associated fungi

To visualize intra-cortical fungi using non-vital stains, root pigmentation and cellular constituents need to be clarified prior to staining. All roots examined were cleared and stained using a modification of the method described by Brundrett et al. (1996a). Fibrous roots were placed into scintillation vials, then immersed in 10% KOH (w/v) and heated in an oven at 95 °C until most cellular contents and pigments were removed. After rinsing with tap water, all roots except maize received an additional clearing with acidified bleach (1.7% NaOCl with 1% diluted HCL v/v in water) at room temperature for more complete removal of recalcitrant pigments. Total bleaching time varied among samples, continuing until roots became noticeably lighter in color; however, bleaching never exceeded 90 s. Following bleaching, roots were rinsed again with tap water, then stained with 0.05% (w/v) trypan blue in lactic acid–glycerol (1:2:2, lactic acid–glycerol–water). Maize and tomato root samples were covered with stain and incubated in an oven at 95 °C for 10 min, while other root samples were heated with stain for 12 min. Stained roots were rinsed and then stored in lactic acid–glycerol for at least 24 h to remove excess trypan blue before examination.

Fungal colonization in roots was observed with an Olympus SZH10 stereo microscope using dark-field illumination, and measured using the line intersect method (Giovannetti and Mosse, 1980). Previously cleared and stained roots were spread on a Petri dish onto which a grid of straight lines was scored so that 1.27 cm (0.5 in.) squares were formed. The grid of lines was used as a reference, where intersections between lines and the root sample were examined for evidence of infection, from which the proportion of infected tissue was derived. When structures of questionable identity were encountered, root sections were removed from the Petri dish, mounted on a slide, and observed in more detail under an Olympus BX51 compound microscope using brightfield and differential interference contrast (DIC) optics.

2.4. IP sample preparation

The IP assay is a modification of the mean infection potential assay described by Moorman and Reeves (1979). This assay provides a relative estimate of fungal propagule infectivity by limiting duration of early interactions between plant host and soil fungi and thus mainly measuring primary colonization of roots. Composite soil samples from individual plots were sieved and fibrous roots from the samples were cut into small pieces (<2 cm) and mixed with the soil. This mixture was divided in half, with one half used to establish pot trap cultures, and other half used for the IP assays. The IP soil was left undiluted and distributed among six 150 mL plastic pots, seeded with maize (*Zea mays* L.), and thinned to one plant after emergence. Pots were placed in trays that were randomly rearranged weekly. Plants were watered daily but not fertilized at any time. Maize plants were harvested 30 days after emergence, at which time roots were excised, cleaned, and dried at 70 °C. A 0.03–0.05 g subsample was cleared, stained, and mycorrhizal colonization estimated by the line intersect method.

2.5. Spore extraction and examination

The soil designated for trap cultures was divided between two 450-mL pots. Five sudangrass (*Sorghum sudanense*) seeds were planted and then watered into the soil in each pot, later thinned to three plants. Pots were randomly rearranged biweekly through the first 6 months of growth, and were fertilized weekly using 35 mL of a Hoagland solution with a very low concentration of P (0.25 mM) after nutrient deficiency symptoms first developed. The plants were allowed to naturally senesce, and were harvested 7 months after planting.

Spores were extracted by an adaptation of methods reported Gerdemann (1955) and Brundrett et al. (1996b). At harvest, roots from sudangrass plants were air-dried, then chopped into fragments (<2 cm) and mixed with the remaining contents of a pot. A 100 cm³ subsample was suspended in 500 mL of deionized (DI) water and filtered through a nested set of sieves with 2 mm (top), 1 mm, and 38 µm (bottom) openings. Filtrate in the bottom sieve was distributed equally among six 50 mL centrifugation vials followed by the addition of equal parts DI water and 50% sucrose (w/v). After centrifugation at 770 × g for 5 min, 20 mL of solution at the sucrose and DI water interface was collected with a syringe, and vacuum-filtered through Whatman 50 paper. Spores were washed from the filter paper into a glass petri dish, examined first under an Olympus SZH10 stereo microscope and then under an Olympus BX51 compound microscope after mounting in polyvinyl-lactic acid–glycerine (PVLG) and Melzer's reagent (1:1 v/v) on slides. Spore specimens with sufficient morphological properties were compared with reference slides and cultures at INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi, West Virginia University, Morgantown) for identification.

2.6. Whole cell fatty acid (WCFA) analysis

The fatty acid 16:1ω5c can be used as a biomarker for AM fungi and the phospholipid fatty acid (PLFA) and neutral lipid fatty acid (NLFA) fractions of 16:1ω5c have been used to estimate AM fungal biomass and levels of energy reserves, respectively (Olsson, 1999). In the present study the WCFA content of 16:1ω5c, which is the sum of 16:1ω5c originating from polar lipids, neutral lipids, glycolipids and free cellular fatty acids, was used to estimate AM fungal biomass in roots and soil. This method is less laborious than the PLFA–NLFA method, which requires lipid fractionation, and has previously been employed both in controlled pot experiments (Madan et al., 2002; Thygesen et al., 2004; Ravnskov et al., 2006; Albertsen et al., 2006; Medina et al., 2007) and in the field (Gryndler et al., 2006).

WCFA were extracted from pulverized root (25 mg) and soil samples (1 g) according to Thygesen et al. (2004). To enable quantification of the extracted fatty acid methyl esters an internal standard, nonadecanoate fatty acid methyl ester (33.7 µg), was

added to each sample. Analysis of fatty acid methyl esters were performed using the software package Sherlock Version 6.0 (MIDI Inc. Newark, Delaware, USA) with a HP Chemstation (Hewlett Packard, Palo Alto, CA, USA) and a HP6890 GC fitted with a 25 m fused silica capillary column (HP part No. 19091B-102) and hydrogen as carrier gas. The injector temperature was 250 °C and the detector temperature was 300 °C. The temperature program was as follows: initial temperature 170 °C increasing to 270 °C at 5 °C/min. Two microlitres per sample were injected. The MIDI software automatically controlled all gas chromatography operations including calibration, subsequent sample sequencing, peak integration, and naming. Calibration standards contained a mixture of straight chain saturated and hydroxy fatty acid methyl esters with a length of 10–20 carbon (MIDI Part No. 1200A).

2.7. Statistics

One-way analysis of variance was used to test for differences in fungal colonization among treatments. Means determined to be significantly different ($P < 0.05$) were separated using Tukey's HSD test. Pearson's correlations were used to test relationships between WCFA results from soil and roots and fungal colonization levels in the IP and field plants, respectively, as well as interactions among endophytes and between endophytes and edaphic factors. Statistical analyses were performed using SAS release 8.02 for Windows.

3. Results

3.1. Edaphic conditions at the study site

Results from soil analyses performed in years four and five of the study are presented in Table 2. Soil P levels were high (794–1831 kg ha⁻¹) due to the fertilization history and nutrient applications made subsequent to the initiation of the land management treatments. Relatively higher levels of this nutrient occurred in the organic treatment where annual additions of BL were made at the rate of approximately 350 kg of applied P ha⁻¹. During seasons of tomato production, the plots in other treatments received a comparatively conservative 25 kg ha⁻¹ of applied P.

Organic matter levels were highest in the organic plots, the only treatment amended with UPD, broadcast at a rate of approximately 70 Mg ha⁻¹ and spread concurrently with BL. The bahiagrass pasture and weed fallow treatments both had long-term stands of vegetation, resulting in substantial carbon input through root deposition, and higher organic matter levels than either the conventional or disk fallow plots.

Flooding from hurricanes in year five of the study, destroyed the initial establishment of raised beds in all but the organic plots, where bed structure was largely retained. Waterlogged conditions persisted long enough in the field to impact soil nutrient

Table 2

Results of soil analyses from year four and five of the 5-year cropping systems study (means ± S.E.).^a

Year	Treatment	OM (%)	P (kg ha ⁻¹)	Bulk density (g cm ⁻³)	pH	S (kg ha ⁻¹)	Mn (kg ha ⁻¹)
4	Bahia pasture	0.75 (±0.04)	795 (±55)	Not measured	7.14 (±0.09)	77.9 (±7.9)	23.2 (±1.2)
	Conventional	0.60 (±0.04)	821 (±51)	Not measured	7.60 (±0.16)	68.0 (±18.4)	23.5 (±2.1)
	Disk fallow	0.53 (±0.03)	819 (±58)	Not measured	7.70 (±0.07)	44.2 (±5.6)	25.6 (±1.1)
	Organic	1.70 (±0.14)	1220 (±90)	Not measured	6.88 (±0.07)	78.0 (±11.4)	37.4 (±2.6)
	Weed fallow	0.82 (±0.02)	884 (±51)	Not measured	7.40 (±0.06)	58.2 (±6.1)	26.8 (±1.4)
5	Bahia pasture	0.73 (±0.05)	794 (±36)	1.43 (±0.02)	7.04 (±0.10)	24.2 (±7.2)	39.5 (±2.3)
	Conventional	0.55 (±0.03)	849 (±35)	1.55 (±0.02)	7.47 (±0.10)	42.3 (±9.0)	11.3 (±3.0)
	Disk fallow	0.44 (±0.02)	899 (±31)	1.55 (±0.02)	7.65 (±0.04)	27.6 (±4.8)	40.3 (±1.2)
	Organic	2.36 (±0.25)	1831 (±80)	1.24 (±0.02)	7.10 (±0.03)	13.6 (±10.3)	76.3 (±2.7)
	Weed fallow	0.69 (±0.02)	859 (±52)	1.47 (±0.02)	7.34 (±0.07)	17.5 (±8.7)	47.9 (±2.8)

^a Results are means of composite samples taken from both halves of treatment plots for each year.

Table 3

Field tomato root colonization by AM fungi and other fungal root endophytes from year four and five of a 5-year cropping systems study.

Year	Orientation	Treatment	AM fungal % colonization ^a	Other fungal root endophyte % colonization ^a	<i>M. bolleyi</i> % colonization ^{a,b,c}
4	South	Bahia pasture	24.1 (±3.5)a	Not measured	Not measured
		Conventional	8.2 (±3.4)b		
		Disk fallow	8.8 (±1.8)b		
		Organic	8.2 (±2.3)b		
		Weed fallow	14.4 (±3.5)b		
<i>P</i> values Treatment			0.0039		
5	North	Bahia pasture	32.6 (±4.6)a	52.1 (±2.0)a	0.6 (±0.4)
		Conventional	9.2 (±1.6)b	39.6 (±1.1)bc	0.1 (±0.1)
		Disk fallow	9.9 (±2.5)b	47.9 (±5.7)ab	ND
		Organic	6.7 (±1.7)c	44 (±1.4)bc	12.8 (±2.2)
		Weed fallow	12.7 (±1.8)b	37.9 (±5.0)c	ND
	South	Bahia pasture	30.3 (±2.6)a	58.4 (±4.8)a	0.5 (±0.4)
		Conventional	15.2 (±2.4)b	47.8 (±5.4)bc	ND
		Disk fallow	11.7 (±1.4)b	48.8 (±6.3)ab	ND
		Organic	4.3 (±0.9)c	37.5 (±1.6)bc	13.0 (±3.1)
		Weed fallow	13.0 (±2.0)b	36.2 (±3.3)c	ND
<i>P</i> values					
Treatment			<0.0001	0.0083	<0.0001
Orientation			0.4011	0.5263	0.7934
Interaction			0.1541	0.2828	0.9856

^a Proportion data were transformed to the square root of arcsine prior to performing ANOVA; values in table are actual mean proportions of root colonization of treatments ± SE. Means followed by different letters were significantly different ($P < 0.05$) using Tukey's HSD test.

^b The identity of the *M. bolleyi*-like fungus was not confirmed by cultivation or molecular methods.

^c ND indicates treatments in year five where *M. bolleyi* was not detected.

availability, decreasing extractable Sulfur (S), and increasing Manganese (Mn).

3.2. Colonization of field roots by AM and other endophytic fungi

3.2.1. AM fungi in tomato and cover crop roots

Tomato root colonization by AM fungi was very low to moderately low across treatments, with significantly higher

levels occurring in the bahiagrass pasture plots in both years, and significantly lower levels of colonization in the organic treatment compared to all other treatments in year five (Table 3). The colonization levels observed in year-five tomato roots were corroborated by WCFA analysis with a positive correlation between mean colonization levels within plots and the 16:1ω5c content measured from root samples ($r = 0.74$, $P < 0.05$).

Table 4

Field soil infection potential of AM fungi and other fungal root endophytes on maize from year four and five of a 5-year cropping systems study.

Year	Orientation	Treatment	AM fungal % colonization ^a	Non-AM fungal root endophyte % colonization ^a	
4	North	Bahia pasture	23.4 (±1.7)ab	52.2 (±1.2)a	
		Disk fallow	4.3 (±0.8)c	29.6 (±3.8)c	
		Organic	14.8 (±2.3)b	46.6 (±3.3)ab	
		Weed fallow	21.7 (±2.5)ab	53.1 (±3.8)a	
	South	Bahia pasture	26.5 (±2.3)a	54.9 (±3.3)a	
		Conventional	24.0 (±1.5)a	40.0 (±3.1)b	
		Disk fallow	25.4 (±5.7)ab	34.4 (±4.5)bc	
		Organic	18.0 (±2.0)ab	28.4 (±3.0)c	
		Weed fallow	21.2 (±3.5)ab	45.4 (±6.5)ab	
P values					
Treatment			0.0040	0.0001	
Orientation			0.0018	0.1668	
Interaction			0.0010	0.0739	
5	North	Bahia pasture	16.9 (±2.5)a	48.7 (±2.9)a	
		Conventional	8.3 (±1.8)b	33.3 (±6.9)b	
		Disk fallow	8.8 (±0.7)b	29.8 (±3.1)b	
		Organic	17.1 (±1.7)a	19.7 (±2.5)c	
		Weed fallow	8.8 (±1.2)b	34.2 (±3.0)b	
	South	Bahia pasture	18.1 (±4.4)a	47.6 (±4.7)a	
		Conventional	9.0 (±1.5)b	28.7 (±6.1)b	
		Disk fallow	9.7 (±1.4)b	30.7 (±3.1)b	
		Organic	22.7 (±4.9)a	17.6 (±1.8)c	
		Weed fallow	7.6 (±1.3)b	38.9 (±3.0)b	
P values					
Treatment			<0.0007	< 0.0001	
Orientation			0.8685	0.8300	
Interaction			0.1571	0.7773	

^a Proportion data were transformed to the square root of arcsine prior to performing ANOVA; values in table are actual mean proportions of root colonization ± SE. Means followed by different letters were significantly different ($P < 0.05$) using Tukey's HSD test.

Colonization levels of AM fungi were also determined for cover crop roots in the northern half of year-four plots, which were rotated into tomato production the following year. Mean root colonization levels were 17.2 and 21.8%, respectively, for bahiagrass roots from pasture and ragweed roots from the weed fallow plots. Sunn hemp roots from organic plots were colonized to a lesser extent, with mean levels among organic plots only reaching 3.4%.

3.2.2. Tomato root colonization by non-AM fungal endophytes

The extensive occurrence of non-AM endophytic fungi was noted in year-four tomato roots, and measured in year-five roots (Table 3). Quantification of these organisms was also determined through the IP assay using soil from treatments in both year four and five (Table 4). When considered as a discrete group, the levels of infection of tomato roots in year five by these other fungal endophytes were considerably greater than that of the AM fungi in the same year. In the season that both groups of fungi were quantified from field roots, their occurrence was positively correlated ($r = 0.47$, $P < 0.0005$). Similar observations were made in the infection potential study and are discussed below.

Phialophora spp. were the most commonly identified fungi within field roots, as determined by the presence of distinctive, enlarged fungal cells within cortical tissue. The hyphae associated with these structures were only occasionally pigmented, although in individual roots the extent of naturally pigmented hyphae was considerable. *Polymyxa* spp. are endophytes within the order Plasmodiophorales, and were found on several occasions in tomato roots, easily recognized by the clusters of resting spores formed within the cortex.

The stimulus for examining the occurrence of a broader range of fungal endophytes than just AM fungi were distinctive structures found within tomato roots produced by what appeared to be *Microdochium bolleyi* (R. Sprague) de Hoog & Herm.-Nijh (Fig. 1). After clearing tomato roots, these masses were the most clearly distinguishable structures where they occurred, irrespective of staining. Tomato roots in the organic treatment were unique in that they were the only roots in this study in which significant levels of this organism was measured, a phenomena that occurred both years, although only measured in year five (Table 3).

3.3. IP assays on greenhouse-grown maize

3.3.1. AM fungal infective inoculum

Soil sampled from both halves of system plots produced generally uniform levels of colonization on maize roots in year

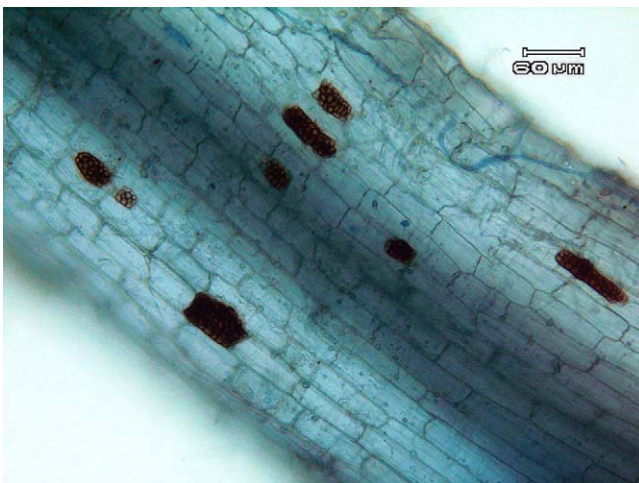


Fig. 1. Pigmented cells formed by *Microdochium bolleyi*-like fungi within tomato roots.

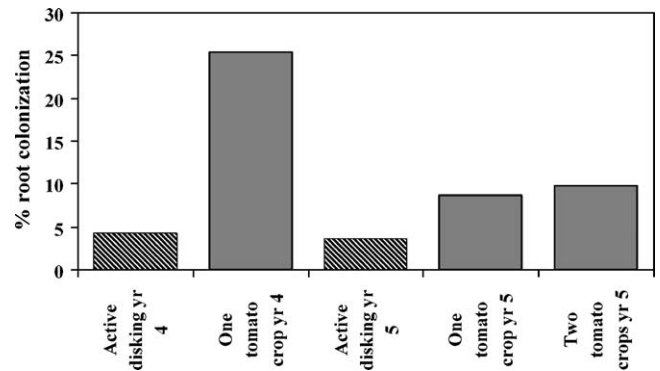


Fig. 2. Infection potential of disk fallow treatment soil sampled in year four and five of the 5-year cropping systems study; infection potential was determined by colonization levels of AM fungi on greenhouse grown maize roots at the five-leaf stage. During this study, the southern half of disk fallow plots produced their first tomato crop in year four and finished a second year of production at the end of year five. Northern plot halves only produced one tomato crop, harvested at the end of year five. Actively disked areas were located in the northern half of plots in year four, and in between plot halves in year five.

four, with the exception being the northern halves of disk fallow plots which differed significantly from all other management strategies (Table 4). The northern portion of the disk fallow treatment was still actively disked at the time of sampling and had been managed to eliminate plant growth for the four previous years. However, after a single full season of host tomato root growth, as occurred in the southern half of disk fallow plots, the IP recovered to the extent that it was indistinguishable from all other treatments (Table 4; Fig. 2). All other treatments were statistically similar, with the exception of slightly lower levels of colonization in the northern half of organic plots at the end of their first year of tomato production when compared to conventional and the southern half of bahiagrass pasture plots.

The IP of all treatments were reduced in year five compared to the previous season, with the exception of organic plots (Table 4). Both halves of the disk fallow plots had been put into production, and at the end-of-season sampling time, they both had inoculum producing similar levels of colonization, albeit reduced from levels that occurred the year before. However, the IP of that portion of plots where disking continued, in between the northern and southern plot halves, colonization levels were almost identical to actively disked soil from the year before (Fig. 2).

Determination of the 16:1ω5 content of field soil helped confirm the IP study results in year five (Fig. 3), and positive

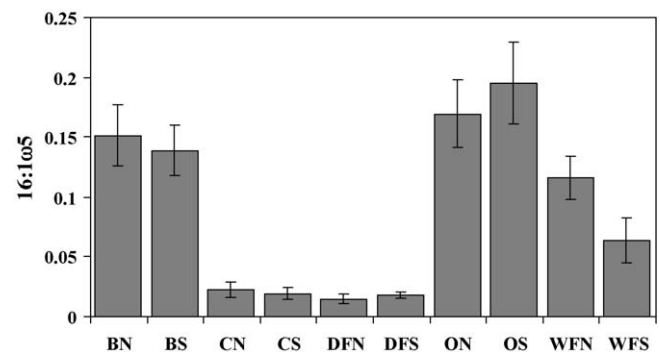


Fig. 3. Results from whole cell fatty acid (WCFA) analysis of soil from year five of the 5-year cropping systems study. Results show the levels of the AM fungal biomarker 16:1ω5 within treatment soil. WCFA content of soil is relative to a fatty acid standard (19:0). The treatments were bahiagrass pasture (B), conventional (C), disk fallow (DF), organic (O), and weed fallow (WF). Treatment codes are followed by an N or S, designating the plot half sampled (N = north, S = south).

Table 5

Diversity of AM fungal morphotypes in year four and five of a 5-year cropping systems study. Morphotypic diversity was determined by extracting spores from sudangrass trap cultures grown in field soil.

Morphotypes extracted	Treatments where morphotype recovered
<i>Acaulospora</i> spp.	Bahia, Conventional, Disk Fallow, Organic
<i>Archaeospora trappei</i>	Bahia, Conventional, Disk Fallow
<i>Gigasporaceae</i> ^a	Bahia, Disk Fallow, Weed Fallow
<i>Glomus ambisporum</i>	Bahia
<i>Glomus clarum</i>	Conventional, Disk Fallow
<i>Glomus etunicatum</i>	Bahia, Weed Fallow
<i>Glomus geosporum</i>	All
<i>Glomus intraradices</i>	Bahia, Conventional, Disk Fallow, Weed Fallow
<i>Glomus mosseae</i>	All
<i>Glomus sinuosum</i>	All
<i>Glomus</i> spp. (orange) ^b	All
<i>Glomus</i> spp. (small hyaline) ^b	All
<i>Glomus</i> spp. (small pale yellow) ^b	All
<i>Glomus</i> spp. (yellow) ^b	All

^a Finer taxonomic designations were not possible with the spore types extracted.

^b These groupings are constructs that could be identified as *Glomus* spp., but could not be classified on a finer taxonomic level; multiple morphotypes may exist within each category.

correlation of these variables at the treatment level was verified ($r = 0.90$, $P = 0.037$). Despite the relatively lower levels of AM fungal colonization that occurred on field plants from the organic plots, it is clear that there was enough active inoculum to cause substantial primary infection on maize in the IP assay. Conversely, bahiagrass pasture plots had the highest levels of field root colonization by AM fungi in year five, but levels of relative infection in the IP assay were not comparably elevated. Maize root infection grown in bahiagrass pasture soil remained significantly higher than levels occurring in conventional, disk fallow and weed fallow treatment soil (Table 4).

3.3.2. Non-AM fungal root endophyte infective inoculum

As in field roots, no negative interaction was detected between AM and other fungal root endophytes co-occurring in IP maize roots. Instead, positive correlations occurred in year four ($r = 0.44$, $P = 0.002$) and year five ($r = 0.20$, $P = 0.117$) with respect to colonization levels of both groups of endophytes within maize roots. Levels of field-root colonization by non-AM fungal endophytes were similar to those occurring in the IP assay (Tables 3 and 4). Bahiagrass pasture soils had the highest overall IP for non-AM fungal endophytes, while organic plots had significantly lower levels of colonization in year five compared to other systems. In addition, there was a general decline in the IP colonization levels in year five compared to the previous year.

3.4. Identification of AM fungal spore morphotypes

There were at least 10 individual morphotypes that were identified as having sporulated between the two sampling dates (Table 5). The majority of spores fell into one of the last four categories on the table, distinguished as *Glomus* spp. morphotypes. Since substantial variation was observed in each group, these categories are constructs for spores that may represent considerable morphotypic diversity, but could not be confirmed to represent different individual morphotypes. Additionally, many of the observed differences in these four groups might be attributable to individual spore morphotypes encountered at different developmental stages or affected by the environment to somehow alter morphological characteristics.

4. Discussion

The diversity of farming strategies among the treatments at the study site occurred across a single soil taxonomic unit, effectively eliminating the substantial influence that soil type and texture can have on microbial communities (Bossio et al., 1998; Girvan et al., 2003). Resilient populations of AM and other fungal root endophytes were found to reside within all systems and no permanent impacts related to anthropogenic alterations or other environmental stresses could be confirmed. However, field-wide suppression of the mycorrhizal association and short-term changes in AM fungal activity related to management practices and flooding were detected.

Of the investigated agricultural practices, P fertilization and soil disturbance had the greatest influences on AM fungal infectivity and abundance, however, their impacts were manifested in distinct ways and persisted over different time scales. The P status of plants has important implications for mycorrhizal colonization and functioning; high levels of available P in the soil result in plant P sufficiency with accompanying higher tissue concentrations and correlative lower levels of plant-associated AM fungal biomass (Koide and Li, 1990; Koide, 1991) and spore production (Douds and Schenck, 1990). Historical and study-associated fertilization practices led to a substantial P bank in all treatments, with the most extreme levels developing in organic plots where P applications were in excess of annual crop need. The abundant available P supply within the soil in all treatments led to a general suppression of AM fungal colonization and probably contributed to a low incidence of sporulation. The large pool of soil P across treatments at the site would represent a long-term obstacle to increased AM fungal development in plants and spore production. Mitigation of the soil nutrient content would require long-term crop production and harvest without significant future P additions.

Tomato roots from the bahiagrass pasture treatment had the highest mean levels of mycorrhizal colonization of any roots observed in this study, reaching a maximum of 32.6% of root tissue colonized. This relatively larger amount of colonization occurring in bahiagrass pasture plots was not due to reduced amounts of available P, but probably to the lack of significant soil disturbance occurring in that treatment. The other treatments all had plot-wide soil mixing followed by the establishment of raised planting beds prior to tomato transplantation and, with the exception of organic plots in year five, this resulted in full-season colonization levels that were not statistically distinguishable from each other.

Tillage-mediated soil disturbance did not have a long-term suppressive effect on AM fungal infectivity, although primary infection was dramatically decreased in soil actively undergoing intensive tillage. In the disk fallow treatment, following 14 years of intensive agricultural management including four consecutive years of continuous soil disturbance, AM fungal inoculum density and infectivity was not significantly impacted compared to the other farming practices investigated after host roots were allowed to persist for a single growing season. This indicates that inoculum can recover from periods of intense disturbance and the absence of host tissue more rapidly than previously suspected (Gosling et al., 2006).

There was no clear evidence that either the history of fumigation at the farm site with methyl bromide and chloropicrin or the continued use of alternative fumigants in the conventional plots had a lasting effect on the ability of AM or other fungal endophyte communities to colonize field roots or to initiate primary infection on maize roots in a greenhouse study. Short-term changes may have taken place in the field however, with repression of fungal populations having taken place directly after fumigation. Results from these studies only included data from end

of season sampling, apparently allowing adequate time for recovery. While analyses of AM fungal signature WCFA's used to measure biomass within soil indicated very low occurrence of these fungi in conventional plots in year five, this method measured similar levels of 16:1w5c within disk fallow plots, suggesting these results may have been largely a consequence of saturated field conditions prevalent in year five rather than the impact of agrichemicals.

While they are aerobic organisms, there is an apparent means through which at least some taxa of these fungi are capable of surviving and functioning in flooded conditions, with saturated soil conditions having a temporary impact on AM fungal activity. Colonization levels have been reported to slow in native wetland plants during periods of flooding, with substantial increases in mycorrhizal development occurring during stretches between flooding (Miller, 2000; Ray and Inouye, 2006). In this study, widespread waterlogging and a shortened growing season in year five may have temporarily reduced the amount and infectivity of all fungal root endophytes as measured by the IP assay, but colonization levels of full season tomato roots were not negatively affected. This seeming dichotomy can be explained. The low incidence of field root colonization by AM fungi in the systems with regularly mixed soil may represent a minimum level of occurrence, as determined mainly by the P status of the host plants and the frequency of disturbance of the soil. The levels of field root colonization were relatively higher in the bahia pasture plots as a result of the spatially dense web of infective hyphae associated with the sod roots. With the exception of AM fungi in the organic treatment, all fungal root colonization levels within soil were reduced in year five compared to year four in the IP assay. This field-wide alteration in the degree of primary infection is most likely due to reduced root growth during a relatively shorter growing season (34 fewer days in year five) in combination with more stressful field conditions, resulting in a reduction of root-associated fungal inoculum.

The significantly lower levels of colonization in year five organic tomatoes may be attributed to soil-related factors, including conditions that both enhance root growth and reduce dependency on mycorrhizal fungi. As established previously the status of the soil P was in excess of plant needs, especially in the organic treatment. Additionally, the lower bulk density and increased aggregate formation that developed with large inputs of organic matter provided a physical environment conducive to root growth by reducing resistance to root extension and improving drainage after waterlogging. The benefits that organic matter lends to the development and maintenance of soil structure were evident after two hurricanes swept over the experimental farm site in 2004. While most treatment plots had their raised beds destroyed, many of the organic plots largely retained their bed structure despite substantial flooding. Reduced colonization levels in these conditions might not be a result of relatively less or decreased infectivity of the inoculum, but rather may be derived from conditions that allowed roots to grow in the organic treatment at a rate that outpaced the ability of AM fungi to develop; a faster rate than occurred in the other treatments. This possibility is supported by the results of the IP assays that show that the levels of primary infection in year five were not comparatively depressed in the organic treatment.

Differentiating among morphotypes described in the INVAM collection requires capturing high-quality spores to visualize incremental variability within groups classified as genera. Cultures generated from undiluted field soil, as in this study, can be problematic for several reasons. Parasitism is extremely common, spore-associated debris can be obscuring and competition among root-inhabiting fungi may cause alterations in AM fungal community spore production and representation. Because of this, and because the adapted harvesting procedure used to simplify

retrieval for mounting rendered pale spores harder to discern, the field diversity of AM fungal spore morphotypes detected in this study must be considered incomplete and may under-represent the richness present at the study site during the 2 years that sampling for identification took place.

There is evidence that AM fungal sporulation is seasonal and influenced by different host plants with considerable variability in intensity of spore production generated among morphotypes (Bever et al., 2001; Liu and Wang, 2003). The detection of morphotypic diversity might be enhanced through spore extractions using soil sampled directly from the field followed by more intensive trapping techniques, especially if assessed at different points within a season and in association with other host plants that were sown or naturally established in plots during this study.

The diversity accounted for, as determined through spore extractions from greenhouse-grown host crops grown in undiluted soil, is not unreasonable for a field with a history of intensive agriculture considering a comparable number of morphotypes have been reported occurring in some surveys of natural and disturbed environments (Douds and Millner, 1999). It is therefore possible to presume that substantial diversity of AM fungal morphotypes has been retained in this field across treatments relative to descriptions from other agricultural environments. Based on the methods used in this study, the organic plots had the fewest positively identified spore types, while bahia pasture contained the most. This is not unexpected considering the abundance of available P in the organic treatment soil, a condition known to contribute to reductions in both colonization and sporulation (Douds and Schenck, 1990; Koide and Li, 1990). *Glomus* spp. spore types were the most common and most frequently identified morphotypes by a large margin in all systems.

Non-AM fungal root endophytes constitute an extensive group of organisms that can be difficult and confusing to distinguish among when attempting to visualize their coexistence within roots. While some fungal structures stain using non-vital dyes and are therefore mistaken for AM fungi in some investigations, others have no pigmentation and do not stain using conventional dyes. This can lead to overestimation of AM fungal colonization as well as inaccurate evaluations of the extent of other endophytic development. The presence of unstained, hyaline hyphae within roots in this study makes it likely that non-AM fungal root endophyte colonization levels were underestimated; the *M. bolleyi*-like endophyte that was predominant in tomato roots harvested from organic plots, undoubtedly suffered from this limitation. The diagnostic structures for this organism as it develops within the plant cortex have been called alternatively, chlamydospores, dark cells, or microsclerotia, and are known to preferentially occupy the inner and outer cortex (Murray and Gadd, 1981; Kirk and Deacon, 1987; Sieber, 2002). While these structures were obvious within roots when the organism produced them, they appeared to be a small portion of the total biomass produced, as extensive colonization occurred through hyaline hyphae that did not stain with trypan blue. The unique occurrence and extensive development of this saprophytic endophyte in organic tomato roots was probably due to the large organic matter inputs and production of a grass cover crop directly preceding tomato transplantation.

There was no evidence of the *M. bolleyi*-like fungi in IP maize roots, although this does not preclude the presence of this endophyte. The maize roots were quite young at the time of harvest and *M. bolleyi* is known to preferentially occupy naturally senescing root cells, generally producing the darkly pigmented diagnostic structures where the plant forms papillae (Kirk and Deacon, 1987). The morphological structures of the other fungal root endophytes observed in maize were similar to those seen in tomato roots, with the exception that extensive occurrence of chytrid *Olpidium* spp. were found only in maize.

Non-AM fungal root endophytes were more abundant and infective than AM fungi in all treatments. With the exception of the nearly exclusive occurrence of *M. bolleyi*-like fungi in roots from organic plots, there were no dramatic differences in the types or levels of non-AM root endophyte colonization in end-of-season field plants or infection potential host roots that could be confirmed among treatments. In addition, no antagonism was detected between AM and other endophytic root fungi with regards to their ability to colonize plant roots, as root co-occupancy was positively correlated.

5. Conclusion

This study has demonstrated that AM fungi are capable of persisting in and recovering from land management practices that are widely considered detrimental to their activities. The impact on the ability of these fungi to form mycorrhizal associations with host plants depended on the time-scale over which the effects of management practices endured. In this subtropical environment, management tactics that created conditions that persisted in the soil, such as abundant available P from fertilization, resulted in a field-wide suppression of colonization while soil tillage had only a transitory effect on the IP and capability of AM fungi to colonize host roots. As a group, non-AM fungal root endophytes were also persistent under intensive agricultural management. Results indicated that the conditions that favored higher infectivity were the same for these endophytes and AM fungi. Future work should consider whether these groups of fungi share similar capacity for resisting stressful conditions in other climatic and edaphic environments as was demonstrated in this study.

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